

CLAIMS

1. A method of producing EndostatinTM protein comprising:
recombinantly producing EndostatinTM using an expression
5 system;
recovering EndostatinTM; and,
purifying EndostatinTM.
2. The method of Claim 1, further comprising
10 lyophilizing EndostatinTM.
3. The method of Claim 1, wherein the expression system is *Pichia pastoris*, yeast, *E. coli*, insect cells, baculovirus, transgenic animals, or transgenic plants.
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4. The method of Claim 1, wherein the expression system is *Pichia pastoris*.
5. The method of Claim 1, wherein the recombinantly
20 produced EndostatinTM has an amino acid sequence shown in SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or SEQ ID NO: 11, or a fragment thereof.
6. The method of Claim 1, wherein the recombinantly
25 produced EndostatinTM is encoded by a nucleotide sequence shown in SEQ ID NO: 4 or SEQ ID NO: 10, or a fragment thereof.
7. A method of recombinantly producing
30 EndostatinTM protein comprising:
preparing an inoculum culture; and
fermenting the culture.

8. The method of Claim 7, wherein the inoculum culture is a two stage seed process of *Pichia pastoris*

5 9. The method of Claim 7, wherein the fermenting step includes fermentation media comprising calcium sulfate, potassium sulfate, magnesium sulfate, potassium hydroxide, phosphoric acid and glycerol

10 10. The method of Claim 7, wherein the fermenting step comprises a fermentation process comprising a batch glycerol phase, a fed-batch glycerol phase, a methanol ramp phase and methanol induction phase.

15 11. A method of purifying EndostatinTM protein comprising:
capturing EndostatinTM from a sample using a first cation exchange column and expanded bed chromatography;
applying the EndostatinTM to a heparin-sepharose column or to a column containing a resin useful for hydrophobic interaction chromatography;
20 applying the EndostatinTM to a an anion exchange column;
applying the EndostatinTM to a second cation exchange column;
and,
concentrating the EndostatinTM.

25 12. The method of Claim 11, wherein the resin useful for hydrophobic interaction chromatography is phenyl sepharose resin.

30 13. The method of Claim 11, wherein the anion exchange column is an amine column.

14. The method of Claim 11, wherein first cation exchange column contains Streamline sulfopropyl resin or carboxymethylcellulose.

5 15. The method of Claim 11, wherein concentrating the Endostatin™ further comprises pushing the sample through a membrane containing a molecular weight cutoff selected for Endostatin™ and eluting Endostatin™ from the membrane with buffer.

10 16. The method of Claim 15, wherein the eluted Endostatin™ is lyophilized.

15 17. The method of Claim 15, wherein the membrane is made from polyethersulfone.

 18. The method of Claim 11, wherein concentrating the Endostatin™ further comprises use of parallel flow concentrators.

20 19. The method of Claim 15, wherein the buffer comprises a citrate-phosphate buffer.

25 20. The method of Claim 19, further comprising removal of citrate by exchanging with phosphate buffered saline and a detergent.

 21. The method of Claim 20, further comprising lyophilizing Endostatin™.

30 22. The method of Claim 21, further comprising reconstituting the lyophilized Endostatin™ with a solution.

23. The method of Claim 22, wherein the solution is an aqueous zinc chloride solution.